On the Oxidation Processes of the Echinoderm Egg during Fertilisation.

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I. Introduction.

The following paper is concerned with an investigation of the oxidation processes of the animal egg-cell during fertilisation. The subject has already received considerable attention and the problem has been approached from many different aspects. The first to attempt to measure in definitive quantitative manner the oxygen consumption of the egg on fertilisation, was Warburg (1) in 1908. He made use of the sea-urchin *Arbacia*, and estimated the amount of oxygen that had disappeared from the sea-water in which the eggs had remained for some time. The Winkler titration method was employed. He found that a quantity of eggs that gave a Kjeldahl determination of 28 mgrm. of egg nitrogen, which corresponds roughly to about 4 million eggs, 4–5 c.c. of oxygen was taken up in the first hour following fertilisation, while the same quantity of unfertilised eggs only consumed 0·5–0·7 c.mm. of oxygen in this time. The fertilised egg, therefore, took up six to seven times more oxygen than the unfertilised egg. Loeb had previously predicted, that the main function of the sperm in the process of fertilisation was that of setting up a series of oxidations on its entrance into the cytoplasm of the egg. Warburg's work was a remarkable confirmation, therefore, of Loeb's prediction. This first paper was followed up by a long series of papers which have added greatly to our knowledge of the oxidation processes taking place in the egg on fertilisation. In addition, our knowledge has also been greatly extended by the numerous papers of Loeb, and especially the papers of Loeb and Wasteneys (2) in which quantitative measurements were also carried out. In 1911 appeared the large paper of Meyerhof (3) in which the heat liberation was measured and correlated with the oxygen consumption. In all these papers the Winkler method was employed; there are, however, many drawbacks to the use of this method, and in the recent work of Warburg and Meyerhof it has been finally abandoned for the more convenient and accurate manometer. The great advantage of the manometer method lies in the fact that it can be used equally well for both oxygen and carbon dioxide determinations, and that continuous observations can be carried out minute by minute on the respiratory exchange of the material under investigation. Warburg (4) (1915), using this instrument, has recently reinvestigated the respiratory exchange in the egg of the sea-urchin *Strongylocentrotus* during the first 24 hours of development. He found that a quantity of unfertilised eggs that contained 20 mgrm. of egg nitrogen, which corresponds to about 3 million eggs, consumed in 20 minutes 10–14 c.mm. of oxygen at a temperature 23° C. and barometer 760 mm. Hg. The fertilised egg under the same conditions, 10 minutes after the addition of the sperm, consumed 60–84 c.mm. That is 10 minutes after fertilisation the oxygen consumption of the egg was six times that of the unfertilised egg, and that there was already a rise of 500 per cent. in the oxidation rate of the egg in this time. In the sixth hour the oxygen consumption was twelve times that of the unfertilised egg, at 12 hours it was sixteen times, while at 24 hours it was twenty-two times the amount of the unfertilised egg. As Warburg remarks, it is extraordinary that in one and the same cell substance, which receives no addition of fresh material from any external source, we should find, as the result of fertilisation in the course of 24 hours, a rise in its oxidation rate of something like 2000 per cent. On the whole the manometer method seemed to show that there was a much closer agreement between the increase in the respiratory quotient and the appearance of visible structure in the egg, than had been demonstrated in previous work where the Winkler titration method had been employed. In all instances the CO_2 output of the eggs followed closely the oxygen uptake, the respiratory quotient being in the neighbourhood of 0·9. The respiration of a single spermatozoön was found to be about 1500–2000 times less than that of the egg.

In the past season, working at Naples, I have been able to carry the investigation of the problem a step farther, by the use of a special type of the Barcroft differential manometer, in which it was possible to bring about the fertilisation of the eggs in the closed chamber of the apparatus, and so for the first time the measurement of the respiratory exchange during the period the sperm were actually making their way into the egg was rendered possible. The eggs and sperm of *Echinus microtuberculatus* were used.

II. METHODS.

In the previous experiments of Warburg (4) (1915), where the manometer has also been employed, readings were only obtained 10 minutes after the addition of the sperm. The following experiments will show that, by the end of this time, the most important part of the process of the fertilisation of the egg has already taken place. There is a great initial inrush of oxygen into the egg and a corresponding output of CO_2 within the first minute after the addition of the sperm to the eggs. It is clear that the spermatozoön sets up an instantaneous oxidation process in the egg, which is perhaps unparalleled in reactions of the animal cell for its sudden character.

In the type of manometer employed in the following experiments the instrument, when used for ordinary blood-gas measurements is designed to allow a small quantity of potassium ferricyanide, held in a small tube in the stopper of the manometer chamber, to run down and mix with the blood when the chamber itself is slightly rotated, so that a groove in the chamber wall comes to lie opposite the opening of the small tube in the chamber stopper. The instrument was readily adapted to the purpose of the present experiments by replacing the ferricyanide by a drop of fresh sperm. The eggs could then be fertilised within the closed chamber of the apparatus, when both eggs and apparatus were in complete equilibrium with the temperature of the water-bath in which the manometer chambers were submerged.

The calibration of the manometers for oxygen was carried out by the Hoffmann (5) method, and for CO_2 by the liberation in the chamber under calibration of a known volume of this gas from 2 c.c. of a Na_2CO_3 solution. All calibrations were carried out under the conditions holding for the following experiments. The temperature of the water of the thermostat in which the chambers of the manometer were submerged was kept constantly at 14·5° C. by means of a coil of piping inside the bath, through which cold water was kept circulating. This temperature is almost the same as that at which the eggs are normally fertilised in the sea. Moreover, it is convenient, as the solubility of CO_2 in sea-water at this temperature and standard barometric pressure is almost 1, so that the CO_2 will distribute itself evenly throughout both sea-water and the air space of the apparatus chambers, and no correction need be introduced for solubility of the CO_2 in the sea-water on this account.

Warburg (4), in his measurement of the CO_2, has taken elaborate means to determine the actual amount of CO_2 given off, by using bicarbonate-free sea-water and estimating the total alkali reserve of his eggs; it is probable that the use of bicarbonate-free sea-water in itself introduces conditions that render respiration far from normal.

In a number of determinations made on the solubility of CO_2 in sea-water at the pressures obtaining under the conditions holding in the chambers of the manometer in the following experiments, it was found this solubility could be neglected, as it was so small in each case as to be well within the error of the manometer reading.* It is only at tensions of 0·4 mm. pressure, or below this, that a great difference is made in the solubility of CO_2 in sea-water. Tensions of this order never obtained in the chambers of the manometer under the conditions of the following experiments. In the following experiments no special importance was attached to the carbon dioxide measurements beyond determining roughly the relation of the carbon dioxide output to the oxygen consumption. No trouble was taken, therefore, to determine the CO_2 output with any degree of accuracy. It is clear that it follows the oxygen consumption very closely, the respiratory quotient being always in the neighbourhood of 0·9.

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* The change of pressure in the chamber of the apparatus during an experiment was very small, being something of the order of 0·005 of an atmospheric pressure. The effect of this on the solubility of carbon dioxide in the sea-water of the chamber could safely be neglected.

To absorb the carbon dioxide in the oxygen manometer, a drop of KOH was placed in the small cup in the bottom of the chamber stopper, and this was renewed after each experiment.

The manometers were made to clamp on a mechanical shaker, such as that used when the instrument is employed for making blood-gas determinations. The motion of this shaker had to be slowed down very considerably into a very gentle to-and-fro motion, or otherwise considerable injury resulted to the egg-membrane. Any injury to the egg-membrane always results in an immediate and abnormal increase of the oxygen consumption of the egg. It was also found that the conical pointed type of chamber with which the instrument is furnished for blood-gas work was highly unsuitable for egg work. The eggs tend to crowd down in the narrow end of the bottle and there clump together, and fertilise very badly. The conical chamber was therefore replaced in the following experiments by a more or less spherical-ended pattern. In making a determination, invariably 2 c.c. of eggs in sea-water were placed in one chamber, while the same quantity of plain sea-water was placed in the control chamber. The chambers were then attached to the manometer and half submerged in the water of the thermostat; a drop of very dilute fresh sperm was then introduced by a fine pipette into the tube in the stopper of the chamber containing the eggs, through a small opening in the top of the stopper. When the glass plug closing this opening had been replaced, the chambers of the manometer could be completely *submerged in the water of the thermostat with the stop-cocks open. It was left at least* 20 *minutes with the shaker working, to come into complete equilibrium with the temperature of the water of the bath.*

If the eggs are not properly cooled to the bath temperature, or if the sperm, when run down on the eggs, are at a different temperature from the eggs, faulty manometer readings will be obtained. To avoid any such errors in the present experiments, great care was always taken to have the eggs, sperm, and manometer, all at exactly the same temperature as the water of the thermostat tank before commencing an experiment. The eggs and sperm, after being washed several times in clean sea-water, were placed in large open-mouthed bottles suspended in the water of the thermostat. They were taken from these bottles as required for an experiment and were therefore always at the same temperature as the bath water. The pipettes used to measure out the eggs and sperm were always kept in separate bottles of sea-water when not in actual use, these bottles being also suspended in the water of the bath. The manometers, finally, were always kept in position on the thermostat when not in use, so that when required for an experiment they were already thoroughly cooled to the temperature of the bath. As already mentioned, in addition to these precautions 20 minutes was always allowed after the eggs and sperm were placed in the manometer chamber before the stop-cocks were closed and the first reading taken. It was found from preliminary experiments in which plain sea-water was used instead of eggs and sperm, that when these conditions were observed in performing an experiment no cooling effect could be produced, and that, under these conditions, the first reading on the manometer was perfectly accurate and satisfactory. In revolving the chambers on the manometers the stop-cocks of the apparatus were opened and immediately closed a moment later. It was possible that, in the operation of revolving the chambers, they might be slightly loosened on their respective stoppers and so the oil drawn round, and a faulty reading obtained in this way. This was avoided if the stop-cocks of the instrument were open while the chambers were revolved. The chambers of my manometers were so accurately ground on to their respective instruments that I was unable, as a matter of fact, to produce any such error experimentally when I turned the chambers while the instruments were closed. An error of this kind could only be produced by actually forcing the chambers completely loose from their manometers. Observing the foregoing precautions then, at the end of 20 minutes the hands of the manometer stop-cocks could be closed and readings commenced on the unfertilised eggs. The same eggs could then be fertilised by turning the chambers and allowing the sperm to run down on the eggs. There was a small error in this reading due to the presence of the drop of sperm in the small tube in the manometer stopper, but care was always taken to use very dilute sperm so that this error could be neglected. To rotate the chambers, when they were attached to their instruments and the manometers themselves in position submerged in the water of the thermostat, wooden handles were wired on the necks of each chamber. These were long enough to project well above the surface of the water, so that the warm hand or fingers never came in close contact or touched the manometer chamber when they were rotated. Separate manometers were used for the oxygen and CO_2 measurements, the output of CO_2

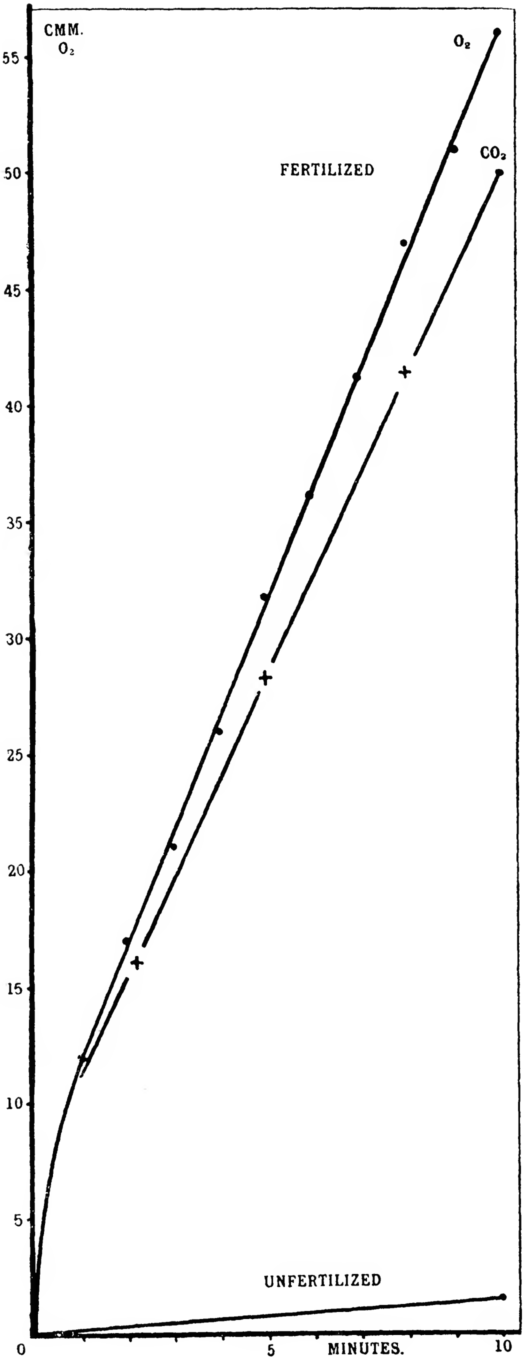

Fig. 1.—Graph showing the amount of oxygen taken up and the carbon dioxide given off during the fertilisation of half a million eggs (4·06 mgrm. of egg N) of *E. microtuberculatus*. The lower line shows the oxygen consumption of the same egg before fertilisation. Broken curve carbon dioxide output of eggs. Respiratory quotient 0·92. The quantities of oxygen and CO_2 are given in cubic millimetres plotted against time in minutes.

being arrived at by deducting the readings of the CO_2 manometer from the oxygen apparatus.

It was inevitable that a considerable difference in the amount of oxygen consumption on fertilisation should have been observed between different batches of eggs and sperm from different females and males. In some individuals the generative products were naturally more ripe and in better condition than in others. As the eggs and sperm for experiments of this kind have to be cut out of the animals and not laid by the animals in the normal manner in the sea, there is always some uncertainty as to their being perfectly mature.

The eggs can only be roughly tested by adding a little sperm to a few eggs in a watch crystal and seeing how evenly and quickly fertilisation takes place. As Warburg (4) remarks, it is obviously necessary in determinations of this kind that fertilisation should take place in all the eggs at the same moment, and that the rate of progress of this process, once it is set up in the eggs, should be the same in all the eggs. These conditions are not always successfully hit off in an experiment. In a certain small number of experiments out of some 200 to 300 performed, these conditions were probably as favourable as the experimental conditions would allow, and in these the readings were remarkably similar. In the following section the figures of three typical experiments of this kind are given. A graph of the readings is shown in figs. 1, 2, and 3, where the oxygen consumption in cubic millimetres is given plotted against the time in minutes.

III. EXPERIMENTS.

Unfertilised Eggs. (20 mgrm. egg N.)

The oxygen consumption of the unfertilised egg is so low that it is quite unmeasureable with any degree of accuracy, unless a much larger number of eggs is used than when a determination is made on fertilised eggs in the ordinary way. Instead of using one to half a million eggs, 4 to 5 millions (20 mgrm. egg nitrogen) were used in arriving at the correct estimation of the respiration of the unfertilised eggs.

The average (15 determinations) amount of oxygen taken up by 4 to 5 million unfertilised eggs, in 10 minutes, under the conditions of the experiments was 7 c.mm. oxygen; at this rate, half a million eggs (4 mgrm. of egg nitrogen) would consume 1·5 c.mm. in this time.

Fertilised Eggs.

Oxygen Manometer. (4·06 mgrm. egg N.)

1st minute	after addition of sperm	12 c.mm.	oxygen consumed.
2nd	„ „	17·0 c.mm.	oxygen consumed.
3rd	„ „	20·0	„ „
4th	„ „	26·0	„ „
5th	„ „	32·2	„ „
6th	„ „	36·0	„ „
7th	„ „	41·4	„ „
8th	„ „	46·8	„ „
9th	„ „	50·0	„ „
10th	„ „	56·0	„ „

Carbon Dioxide Manometer. (4·08 mgrm. egg N.)

At end of	2	minutes after addition of sperm difference was	2 c.mm.
„	5	„ „ „	4 „
„	8	„ „ „	5 „
„	10	„ „ „	6 „

At the end of the experiment the manometer chambers were opened, when it was found that the eggs in both chambers had all formed normal fertilisation membranes and consequently all formed regular two-cell segmentation stages. A Kjeldahl determination on the eggs showed that in the case of the eggs in the oxygen chamber 4·06 mgrm. of egg nitrogen were present, and in the case of the CO_2 chamber 4·08 mgrm. of egg nitrogen were present.

It will be seen from the foregoing Tables and graph, fig. 1, that on addition of the sperm to the eggs there is an immediate consumption of oxygen. In the course of the first minute the uptake of oxygen is many times that of the same eggs one minute before the addition of the sperm, and more is usually taken up in the first minute than is taken up in the second and third minutes after the addition of the sperm taken together.

In all instances the CO_2 output of the eggs follows the oxygen uptake very closely, the respiratory quotient being in the neighbourhood of 0·92.

In fig. 2 is shown a graph of another experiment similar to the former, in which half the quantity of eggs were employed (2·08 against 4·06 mgrm. egg nitrogen). It will be seen both curves are similar, the later being half the value of the former.

At standard barometric pressure, and temperature of 14·5° C., 4·06 mgrm. of egg nitrogen (half a million eggs) which before fertilisation consumed

1·5 c.mm. of oxygen in 10 minutes, after fertilisation consumed 56 c.mm. in this time; there was thus an increase in the respiratory quotient of the eggs in 10 minutes after fertilisation of something like 37 times that of the unfertilised condition. If we consider the increase taking place at the end of the first minute after the addition of the sperm to the eggs, we get

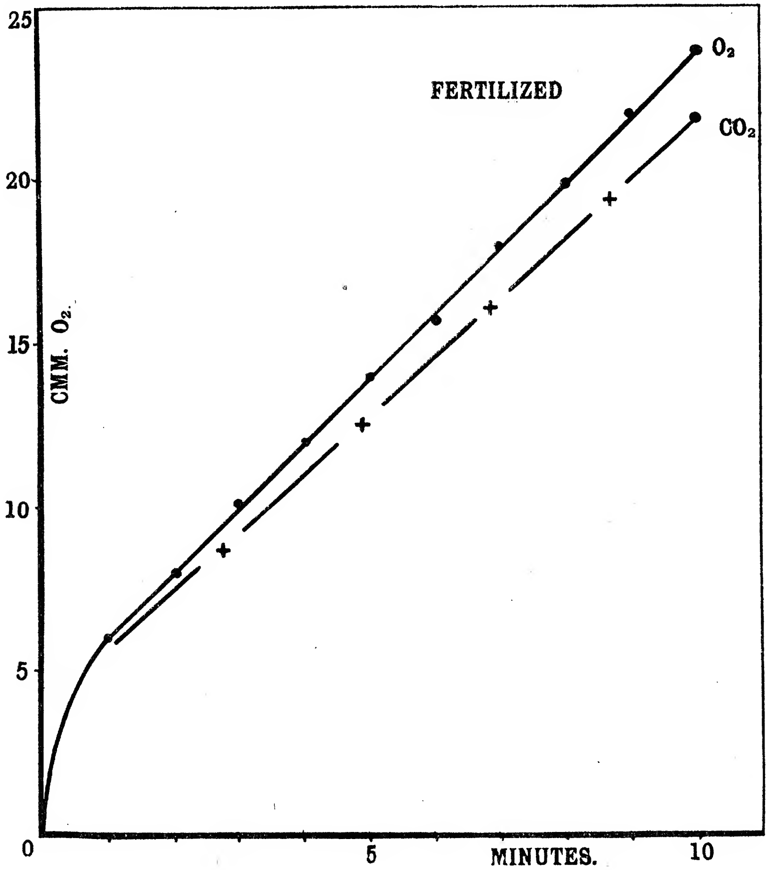

Fig. 2.—Graph showing the amount of oxygen taken up and the carbon dioxide given off during the fertilisation of a quarter of a million (2·08 mgrm. egg N.) eggs of *E. microtuberculatus*. It will be seen that this graph is similar in all respects with that shown in fig. 1, except that it is half the size of the former, half the quantity of eggs being used.

even more striking figures. The oxygen consumption of the unfertilised egg is, we have seen (15 determinations), about 1·5 c.mm. oxygen for 4 mgrm. of egg nitrogen in 10 minutes, and in the case of the present experiment a reading of this value was actually obtained on the unfertilised egg. If we divide this figure by 10 we arrive at the value of the oxygen consumption for 1 minute = 0·15 c.mm. The same eggs fertilised consumed in the first minute after the addition of the sperm 12 c.mm. of oxygen. Thus, the addition of the sperm to the eggs causes, within the space of 1 minute, an increase in their oxygen consumption of something like 80 times that observed on the same eggs 1 minute previous to the addition of the sperm.

The examination of sections of fixed material of the eggs of *E. microtuberculatus* during different stages of the process of fertilisation shows that the sperm take at least 10 to 15 minutes to enter the cytoplasm of the egg. In material fixed within 2 minutes of the sperm being added to the eggs, the sperm are seen only attached to the external surface of the egg membrane. They have not penetrated the membrane itself.

This initial oxygen consumption of the egg immediately on fertilisation must be brought about by the first contact of the sperm with the external surface of the egg membrane. We arrive then at the remarkable conclusion that contact of the spermatozoön with the external surface of the egg is capable of increasing its oxygen consumption in 1 minute by something more than 8,000 per cent. In fact, the total oxygen consumption of the eggs shown in figs. 1 and 2, represents some change brought about in the egg by the spermatozoön before it has entered the egg, and before it has formed a male pronucleus in the egg cytoplasm. It is moreover clear, that when the fusion of the male and female pronucleus takes place at a later stage of the process, it is not accompanied by any fresh rise in the oxygen consumption of the ovum, but instead, a slight drop in the curve is often observed about this time (see fig. 3). The nuclear features of syngamy therefore, seem connected in no direct way with the oxidations taking place in the ovum during fertilisation.

In fig. 3 is shown a graph of an experiment which illustrates these points. It represents the amount of oxygen taken up and the carbon dioxide given off during the fertilisation of a quantity of eggs that contained 2·8 mgrm. of egg nitrogen. The curve extends over a period of 1 hour after the sperm were added to the eggs, and so to a time when the first segmentation division has been completed and the egg has attained the two-cell stage. The readings are in 2-minute intervals up to the end of 10 minutes, and after this at 5-minute intervals. At the end of 2 minutes 10·5 c.mm. of oxygen had been consumed, which compares favourably for this quantity of eggs, with the figures obtained in the previous experiments, the graphs of which are shown in figs. 1 and 2.

It will be seen that at the end of 15 minutes the curve begins to appreciably flatten, and this flattening increases at the 25th minute when the fusion of the pronuclei is taking place. At the 40th to 45th minute it begins to rise again and this synchronises with the first segmentation division. The steepest part of the curve is that in the first 10 minutes interval, which corresponds to the action of the sperm on the external surface of the egg membrane.

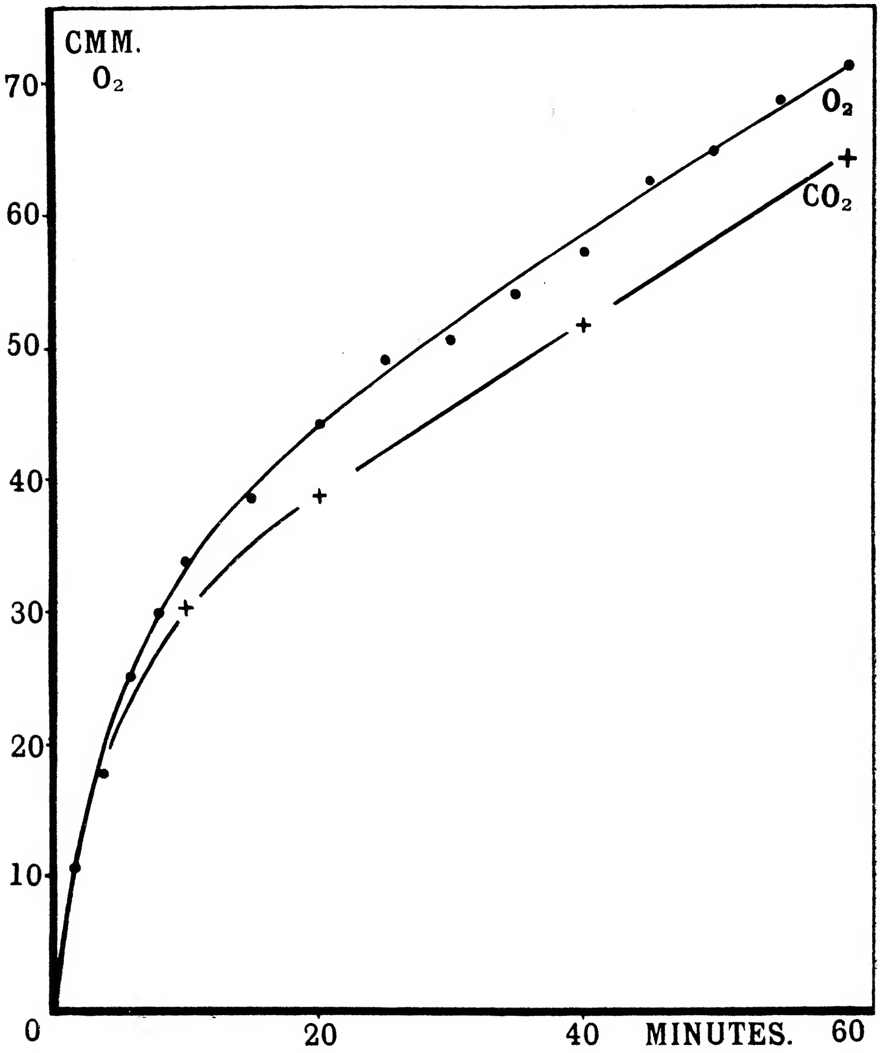

Fig. 3.—Graph showing the oxygen consumption and carbon dioxide output of a quantity of *E. microtuberculatus* eggs that contained 2·8 mgrm. of egg nitrogen during the course of the first hour following fertilisation. It will be seen that the steepest part of the curve is in the first 15 minutes, while the sperm are in relation with the egg membrane. At the 25th minute, when the male and female pronuclei are undergoing fusion, there is a slight flattening of the curve.

As the supply of free energy required by the developing ovum is mainly or entirely derived from its oxidation processes, it is surprising to find that during fertilisation, where a very large amount of energy is immediately rendered available, none of it is liberated as the result of the fusion of the egg pronuclei, a feature which heretofore has always been considered the most essential and important part of the whole process.

It is of interest in this connection to compare roughly the oxygen consumption of the ovum on fertilisation with that of the fixed tissues of the adult body. If we take liver tissue as one in which the metabolic rate is high, according to Barcroft and Shore (6), 1 grm. of fresh liver tissue from a well-fed cat consumes 0·024 c.c. of oxygen per minute, which is equivalent to 240 c.mm. in 10 minutes. If we take Schryver's (7) figures of the protein nitrogen-content of 1 grm. of well-fed cat's liver as 22·4 mgrm. nitrogen, we find the oxygen uptake of the ovum in the first 10 minutes of the fertilisation process, as compared with that of a similar quantity of liver protein in the same time, is in about the ratio of 13·8 to 10·7 in round figures. In the unfertilised egg this proportion is 0·37 to 10·7 for the same time.

IV. Discussion.

There are good reasons for believing, as the result of Loeb's (8) experiments on the fertilisation of the eggs of *Strongylocentrotus* with the sperm of *Asterias*, and Lillie's (9) description of the process of fertilisation in *Nereis*, that the entry of the spermatozoön into the egg consists of two distinct phases. Firstly, an external one, in which certain changes are brought about in the cortical substance of the egg the moment the sperm make contact with the external surface of the egg-membrane; this would seem to be correlated with the initial oxidation taking place in the egg, as described above for *E. microtuberculatus*. Secondly, the changes following the actual entry of the spermatozoön into the egg cytoplasm itself, which, as Lillie has shown in *Nereis*, only takes place some 30 minutes after the first phase of fertilisation, and in the sea-urchin follows some 10 to 15 minutes after the sperm are added to the eggs.

By centrifuging the eggs of *Nereis* before the sperm has actually penetrated the egg-membrane, Lillie was able to separate the jelly surrounding the egg and containing the spermatozoön from the egg itself. These eggs complete meosis, which has been initiated by the spermatozoön, but never segment. A typical segmentation nucleus is, however, formed, which breaks down leaving the chromosomes free in the egg cytoplasm ; they split longitudinally in the normal manner, but never separate. No asters or miotic spindle appear in these eggs, as when the complete process of fertilisation is allowed to take place; and in the absence of these structures the process of cell division makes no further progress, and the chromosomes finally degenerate and break down. This experiment clearly proves that the sperm bring about profound alterations in the egg while still external to the egg-membrane. Loeb (8) has shown that when the eggs of *Strongylocentrotus* are fertilised with the sperm of *Asterias,* in hyper-alkaline sea-water, they only form fertilisation membranes: no actual segmentation takes place unless the eggs receive further treatment so that artificial parthenogenesis is induced.

Meyerhof and Warburg in many of their experiments have shown that any injury or cytolysis of the egg-membrane is invariably followed by a great increase in the oxygen consumption of these eggs. Meyerhof (10) found that this is usually accompanied by an increased liberation of heat. In eggs treated with weak solutions of NaCl, in which the normal condition of the cell-wall is destroyed in the absence of Ca and K ions, the rise of oxygen consumption was five times that of the same quantity of untreated eggs. The heat production was increased from 0·9 grm.-calories per hour to 3·4 grm.-calories per hour, after treatment with valerianic acid, by which artificial membrane formation had been induced.

A great many of Loeb's and Warburg's experiments point conclusively to the cortical layer of the egg and the egg-membrane as being the controlling factor in the oxidation processes of the egg. Any change brought about in these is immediately reflected in the oxygen uptake of the egg. Loeb has, of course, based his method of producing artificial parthenogenesis on the fact that alteration of the surface layer of the egg renders the commencement of development possible. But how can the cytolitic destruction of the surface layer of the egg lead to development? Warburg has shown that there are good reasons for believing that the oxidations taking place in the egg occur mainly at its surface, for NaOH, which does not diffuse into the egg, raises the rate of oxidations more than NH_4OH, which readily diffuses into the egg. Moreover, he found (11) that the addition of iron* salts to the broken up eggs, or acetone egg powder, was followed by a considerable increase in the oxygen consumption of these egg preparations. If the iron-content of the egg powder was doubled, the uptake of oxygen was also doubled. He found marked traces of iron in the sea-urchin egg. He suggests that the iron probably acts the part of a catalyser. If the iron were located in the lipoid layer of the egg in a condition in which it was unable to act, some slight alteration in this layer, due to the action of the sperm, might render it active or bring both the iron and the oxidisable substrate into a condition in which they could quickly interact. We know from Thunberg's work (12) that lecithin in a watery suspension consumes considerable oxygen in the presence of iron salts. The egg of the sea-urchin contains considerable quantities of this lipoid.

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* Warburg found 0·03 mgrm. iron per gramme dried egg substance.

Warburg (13) has pointed out that there are many points in which the metabolism of the fertilised egg resembles that of the yeast cell. In each it has been shown that structure plays a very important part, as acetone preparations of both the egg and the yeast-cell retain considerable respiratory power. Meyerhof (14) finds, however, that if acetone yeast is well washed with water, it soon loses its capacity to take up oxygen. If a little watery extract of yeast is added to the washed yeast, it immediately regains its lost respiratory power. In the water used in washing the yeast Meyerhof found the presence of some compound containing the (SH) group. Hopkins (15) has recently isolated from the yeast cell a substance which is undoubtedly closely related, if not identical with this respiratory body of Meyerhof. It proves to be a combination of two amino-acids, glutamic acid and cystine, to which Hopkins has given the name of glutathione. This dipeptide possesses most remarkable properties in that, in the reduced (SH) form, it can take up molecular oxygen, while in the oxidised (S–S) form so produced it can act as a hydrogen acceptor, and can catalyse oxidations of the Wieland type, in which no activation of oxygen probably takes place, but an activation of hydrogen occurs instead. In the presence of a suitable acceptor the hydrogen is removed and the oxidation of the original substance takes place. It can therefore be both reduced and oxidised under the influence of factors known to be present in the tissues themselves. Moreover, it possesses precisely those properties which a co-ferment adapted to an oxidase system would possess, and at present stands entirely in a class by itself. Hopkins has shown that it is present in most living cells, but he could find no trace of it in the hen's egg, although it was very obviously present in the 30-hour chick.

I find, however, that in the ripe eggs and sperm of *E. miliaris* it is invariably present in an appreciable quantity in the reduced form, but one minute after fertilisation the same eggs give a very deep magenta colour by the nitro-prusside test. It is very readily washed out of the eggs by warming them with a trace of acetic acid in sea-water: the washed eggs then no longer give the test. In the unripe egg, in which the nucleus is plainly visible, I could find no trace of its presence by the nitro-prusside test. In the ripe eggs it is present in the reduced form in very variable quantities, no two females giving the same result, probably depending on varying degrees of ripeness of their gonads. In a number of samples of ripe sperm it seemed to be present in much less quantity than in the eggs; but here again in two samples of sperm it was present in much greater quantity than in any of the eggs examined.

It is, of course, possible that the dipeptide is present in considerable quantity in the eggs and sperm in the oxidised (disulphide) form, and that during fertilisation it undergoes reduction. It has, of course, long been known that the hydrogen ion concentration of the sea-water exercises a marked influence on the uptake of oxygen by the egg of the sea-urchin. Thus Warburg (13) found that an increase in the HO concentration in the sea-water in which the eggs of *Strongylocentrotus* were placed from 10^{-8} to 10^{-3} increased the oxygen consumption of the eggs from 1·4 to 8·1. Hopkins finds that glutathione in the oxidised form is rapidly reduced by fresh tissues, but that this reduction is greatly accelerated if the reaction or P_H of the medium is well on the alkaline side of neutrality, while an acid reaction greatly retards this reduction. In a similar manner the oxygen uptake of the egg-cell is accelerated by alkali and retarded by acids.

That the glutathione is readily washed out of the eggs is shown by a slight pink colour the wash-water gives by the nitro-prusside test. In certain experiments in which the unfertilised eggs were treated so that their glutathione was washed out, I could find no trace of respiratory power on the part of these washed eggs. The same eggs unwashed showed well-marked respiration. The oxygen consumption of the unfertilised egg is so low, however, that it is perhaps unfair to assume on this ground that glutathione is the sole body concerned in the respiration of the ovum. Prof. Hopkins has been so kind as to undertake certain experiments with washed egg material for me, and he tells me that on the addition of glutathione to these egg preparations, the reduction of this in the presence of fresh tissue was markedly greater in the case of the fertilised washed eggs than in the case of the unfertilised. There seems to be fairly substantial ground then for believing that there is an immediate increase in the quantity of this remarkable body in the ovum on fertilisation.

It has been mentioned that cystin is one of the amino-acids entering into the composition of glutathione; it is interesting to note that Warburg (16) has recently drawn attention to the fact that cystin, absorbed on the surface of carbon particles, is capable of considerable respiration, taking up oxygen and giving off carbon dioxide. He found that one gram of blood carbon dissolved in a similar weight of a 1/500 N cystin solution, took up the same quantity of oxygen as a similar weight of liver tissue. The carbon-cystin system, moreover, under the action of oxygen, gives the same end-products as the combustion of egg-white, that is carbon dioxide, ammonia and sulphuric acid.

There are a number of other interesting points brought up by the presence of glutathione in the germ-cells of the sea-urchin and the possible *rôle* it may play in the oxidation processes of the ovum. There can hardly be much doubt, therefore, that its investigation in the future will reveal many new and hitherto unsuspected facts with regard to the oxidations taking place in the ovum on fertilisation.

V. SUMMARY.

1. By the use of a special type of the Barcroft differential manometer the oxygen consumption and the carbon dioxide output of the egg of *E. microtuberculatus* has been measured during the period the sperm are actually making their way into the egg. The eggs were fertilised in the closed chambers of the apparatus and their respiration observed before and during fertilisation.

2. It has been shown that during fertilisation the sperm within a minute of their being added to the eggs bring about an immediate increase in their oxygen consumption.

3. The study of sections of fixed material of the eggs during the process of fertilisation, shows that within 2 minutes of the sperm being added to the eggs they have not penetrated the egg membrane. They are only in contact with its external surface.

4. This contact of the spermatozoön with the external surface of the egg membrane, however, is capable of increasing in the space of a minute the oxidation rate of the ovum by something more than 8000 per cent.

5. During fertilisation there is more oxygen taken up in the first minute of the process than at any subsequent interval of the same time.

6. The carbon dioxide output of the eggs during fertilisation closely follows the oxygen consumption, the respiratory quotient varying from 0·9 to 0·95.

7. The curve of the oxygen consumption of the ovum during fertilisation points conclusively to the stage when the sperm are in contact with the egg membrane as the most important part of the process.

8. The fusion of the male and female pronuclei in the later phases of fertilisation is correlated with no additional increase in the oxygen consumption of the egg-cell.

9. The oxidation rate of the ovum on fertilisation is probably considerably greater than that of any of the adult body tissues, while the oxidation rate of the mature unfertilised ovum is very much less than that of any adult body tissue.

10. The ripe sperm and eggs of *E. miliaris* contain appreciable quantities of the dipeptide *glutathione* in the reduced (SH) form. In the egg 1 minute after fertilisation it is found in much greater quantity than in the unfertilised condition. It can be washed out of both the fertilised and unfertilised egg.

The washed eggs no longer give the nitro-prusside test. In the immature germ cells it seems absent, at least in the reduced form.

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